

Thymineless Death in Recombination Deficient Mutants of *Escherichia coli* K12

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When grown in thymine-free minimal synthetic medium, *thyA recA* mutants of *Escherichia coli* K12 were comparatively more resistant and *thyA recBC* mutants more sensitive to thymineless death (TLD) than their respective parent strains. No excessive numbers of single-strand breaks were observed in the DNA of the *recBC* mutant strain starved for thymine, hence the hypersensitivity for TLD in this strain was not caused by this type of DNA damage. Although experiments were performed with the same *recBC* mutant strain as used by earlier workers, results presented here contradict earlier findings that *recBC* mutants are no more sensitive and *recA* mutants are no more resistant to TLD.

Introduction

If *thy* mutants of *Escherichia coli*, in their exponential growth phase, are transferred to a medium devoid of thymine, they undergo thymineless death (TLD) [1]. Despite vast information available on this unique cellular event, the exact mechanism of TLD is not yet clear. Reported alterations in structure and properties of DNA upon thymine starvation include single-strand breaks, both chromosomal [2] and episomal [3], double-strand breaks [4], decrease in template capacity for RNA polymerase [5], DNA polymerase [6] and DNA methylase [7]. If cells starved for thymine are returned to a thymine supplemented medium, non-conservative DNA synthesis occurs; this was attributed to regional repair of lesions in DNA caused by thymineless state [8].

Strains of *E. coli* having mutations in *pol A*, *lig* and *rep* are more sensitive to TLD than their respective non-mutant parent strains [9–12]. Also *E. coli B* is more sensitive than *E. coli B/r* [13].

TLD in recombination deficient mutants of *E. coli* has been studied in a number of laboratories and diversified data have been presented; thus whereas *E. coli* strain JC 1569 (*thy*, *rec A*) was found to be no more sensitive to TLD than its *rec⁺* parent strain [13], strain mr 2–41 (*thy*, *rec A*) was more stable towards TLD in comparison to *thy* strains [14]. The *rec BC* mutant of *E. coli*, SDB 1318, was found to be

no more sensitive to TLD than its wild type parent strain [15] and strain JC 4457 (*thy*, *rec B*) was less sensitive to this phenomenon in comparison to *thy* strain [16]. In another study *rec C* strain, AB 2470, was super-sensitive to TLD [17]. Thus it appears that *rec* mutants of *E. coli* show various phenotypes with respect to TLD.

To attempt to resolve this discrepancy TLD was measured in a *rec A* and two *rec BC* mutant strains. Data are presented which show that *rec A* cells are comparatively more resistant and *rec BC* more sensitive to TLD in comparison to their *rec⁺* parent strains. Moreover, the high sensitivity of *rec BC* strain is not due to excessive numbers of single-strand DNA breaks. Finally, it was noted that the *rec⁺* and *rec A* cells incubated in rich medium in thymineless condition die faster than the cells incubated in simple minimal medium.

Materials and Methods

Bacterial strains: All strains are listed in Table I.

Growth media: Cells were grown either in nutrient broth (Difco Bacto Nutrient Broth) or in M9 minimal medium supplemented with required amino acids [18] and casamino acids (2.5 g/l w/v) whenever necessary. M9X1 was used to dilute the cultures.

Isolation of thymine requiring mutants: Aliquots of 0.1 ml of overnight grown cells in M9 minimal medium plus thymine (50 µg/ml) were spread on M9 minimal agar plates supplemented with trimethoprim (10 µg/ml) plus thymine (50 µg/ml).

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Table I. Bacterial strains

Strain	Relevant genotype		Origin
	<i>rec</i>	<i>thy</i>	
AB 1157	+	+	ref. [28]
SA 229	+	—	<i>thy</i> derivative of AB 1157
AB 2463	A 13	+	ref. [28]
SA 230	A 13	—	<i>thy</i> derivative of AB 2463
JC 5519	B 21, C 22	+	ref. [28]
SA 231	B 21, C 22	—	<i>thy</i> derivative of JC 5519
HF 4733	+	—	ref. [15]
SDB 1318	B 21, C 22	—	ref. [15]

Other genotypes: AB 1157, AB 2463, JC 5519, SA 229, SA 230 and SA 231: F⁻, *thr-4*, *leu-8*, *pro-2*, *arg-3*, *thi-1*, *his-4*, *mtl-1*, *xyl-5*, *ara-14*, *gal-2*, *lac Y-1*, *strA-31*, *tsx-33*. HF 4733: F⁻, *gal*, *thi* and endonuclease-I deficient. SDB 1318: F⁻, *his*, *gal*, *thi* and endonuclease-I deficient.

Most colonies appearing after 48 h incubation at 37 °C were *thy* mutants.

Measurement of thymineless death: Cells grown overnight in M9 minimal medium supplemented with thymine (20 µg/ml) plus casamino acid (wherever specified) were diluted 20-fold and grown to exponential phase with aeration at 37 °C. A 10 ml sample (1 × 10⁸ cells/ml) was centrifuged, washed once with M9 medium and resuspended in twice the volume of fresh M9 medium without thymine. Cultures were incubated at 37 °C with aeration. At intervals 0.1 ml samples were withdrawn, appropriately diluted and plated on nutrient agar plates supplemented with thymine (50 µg/ml).

Radioisotopic labelling of cellular DNA and sedimentation of DNA through alkaline sucrose gradient: These were carried out using the procedure described elsewhere [19].

Results

TLD in *rec* mutants of *E. coli* was measured in the presence and absence of casamino acids (CAA). Data (Fig. 1) show that the *rec A* cells (SA 230), if incubated in CAA supplemented medium (doubling time 63 min) are slightly more resistant to TLD than those of the *rec*⁺ *thy* strain (SA 229). However, when the *rec A* strain is incubated in medium free from CAA (doubling time 162 min) it shows enhanced resistance for TLD (Fig. 2). Even samples analysed after 6 h starvation showed no increase in TLD from that recorded at 3 h (data not presented).

From Figs 1 and 2 it is obvious that the *thy rec BC* strain (SA 231) whether incubated in the presence of CAA (doubling time 66 min) or absence of this compound (doubling time 96 min), is super-sensitive to TLD. Since this differs from the earlier observation that the *rec BC* strains were no more

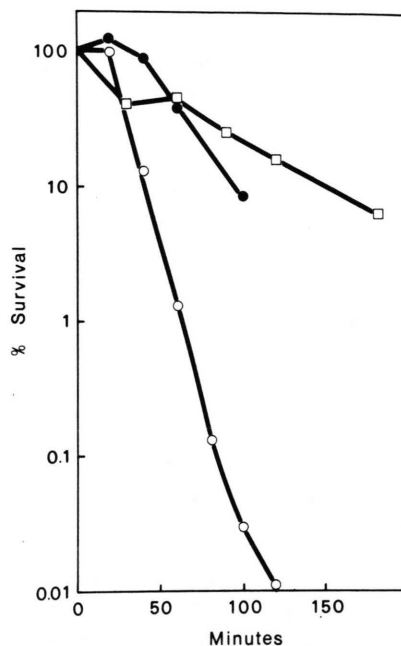


Fig. 1. Thymineless death in *rec A*⁻, *rec B*⁻*C*⁻, and *rec*⁺ strains in the presence of casamino acid; □—□ *rec A*⁻ (SA 230), ○—○ *rec B*⁻*C*⁻ (SA 231); ●—● *rec*⁺ (SA 229).

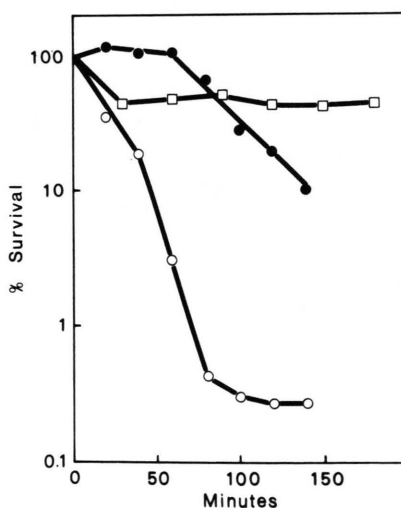


Fig. 2. Thymineless death in *rec A*⁻, *rec B*⁻*C*⁻, and *rec*⁺ strains in the absence of casamino acid; □—□ *rec A*⁻ (SA 230), ○—○ *rec B*⁻*C*⁻ (SA 231), ●—● *rec*⁺ (SA 229).

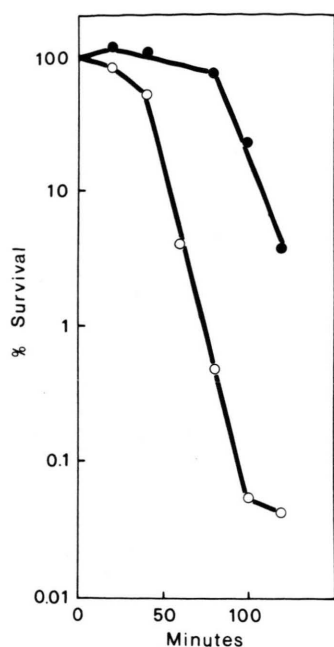


Fig. 3. Thymineless death in ●—● HF 4733 (*rec*⁺) and ○—○ SDB 1318 (*rec B-C*⁻) strains.

sensitive to TLD [15], TLD was measured in the same *thy*, *rec BC* strain (SDB 1318) used by earlier workers. As may be seen even this strain shows greater sensitivity to TLD than its isogenic *thy* strain, HF 4733.

A comparison of the two methods revealed that the earlier workers used thymidine to grow the cells prior to their starvation for thymine [15] whereas in this study thymine was used. In an attempt to grow the cells with thymidine it was observed that this compound unusually inhibited the growth of HF 4733 and SDB 1318 [20]. This fact, however, does not produce a convincing reason for the two results to differ.

To examine if the increased sensitivity of *rec BC* mutants was due to excessive numbers of single-strand DNA breaks, samples of DNA from various strains starved for thymine for 1 h were sedimented through alkaline sucrose gradients. The DNA sedimentation pattern of the *rec BC* strain remains unchanged whether or not the cells were starved for thymine (Fig. 4). From this it is concluded that the increased rate of TLD in this strain is not due to excessive numbers of single-strand DNA breaks.

However, a comparison of data (Figs 4, 5) indicates that the *rec BC* strain has more low molecular weight DNA than *rec*⁺ cells. Also the *rec*⁺ cells starved for thymine show fewer low molecular weight DNA than the cells grown with thymine.

Finally a comparison of data (Figs 1, 2) reveals that the rate of TLD in *rec A* and *rec*⁺ strains, when incubated in M9 minimal medium, is slower than the rate in CAA supplemented medium.

Discussion

Exonuclease V, the product of *rec BC*, in *E. coli* is an ATP dependent exonuclease with a variety of *in vitro* catalytic properties [21, 22]. The hyper-sensitivity of *rec BC* mutants suggests that the exonuclease V plays certain roles in protecting the cells from TLD. In the light of the observation that *rec A rec B* double mutants are unable to repair the large patches in DNA [23], it is proposed that the recombinational repair system operates to repair the DNA damaged during thymine starvation and exonuclease V participates in the process. The hypothesis is further supported by the observation that an ATP-stimulated DNA synthesising system depends primarily on the activities of DNA polymerase I and *rec BC* enzymes; the two activities form a complex and have been isolated and further characterised [24].

An explanation for the hyper-resistance of *rec A* mutants may be that the *rec A* product, the protein X [25], in *rec*⁺ strains, is induced during thymine starvation and this aids to the unbalanced growth and consequently cellular death by inhibiting the formation of septa [26]. Cellular filamentation and unbalanced growth is a well documented phenomenon occurring during thymine starvation and has been related with TLD. This explanation is further supported by the fact that the cells starved for thymine in CAA supplemented medium die faster than the cells incubated in minimal medium free from this compound. Clearly because in CAA added medium the cellular filamentation would occur faster than in CAA-free medium.

The DNA sedimentation patterns of *rec BC* strain show that the increased rate of TLD in this strain is not due to single-strand DNA breaks. However, the *rec BC* strain, whether grown in the presence or absence of thymine, shows more lower molecular

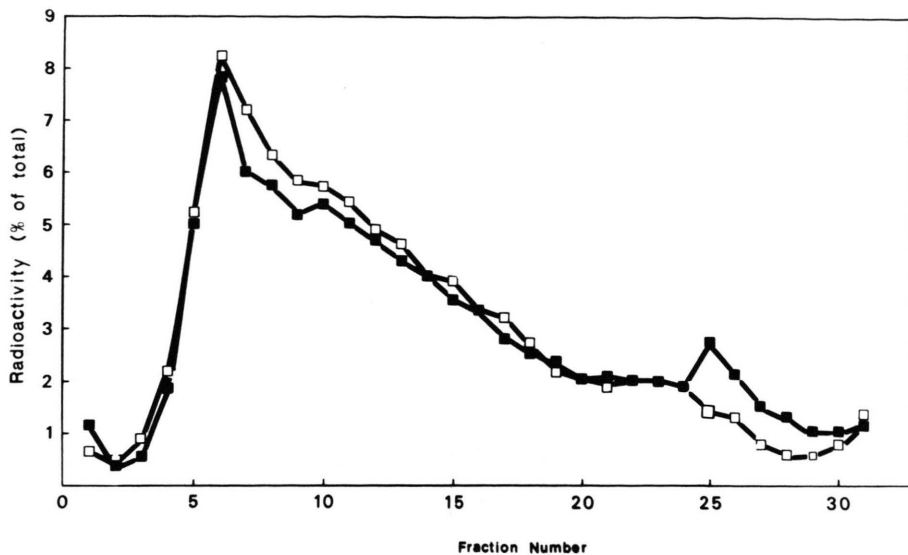


Fig. 4. Alkaline sucrose gradient profiles of DNA from SA 231 (*rec B⁻ C⁻*): ■—■ starved of thymine, □—□ unstarved.

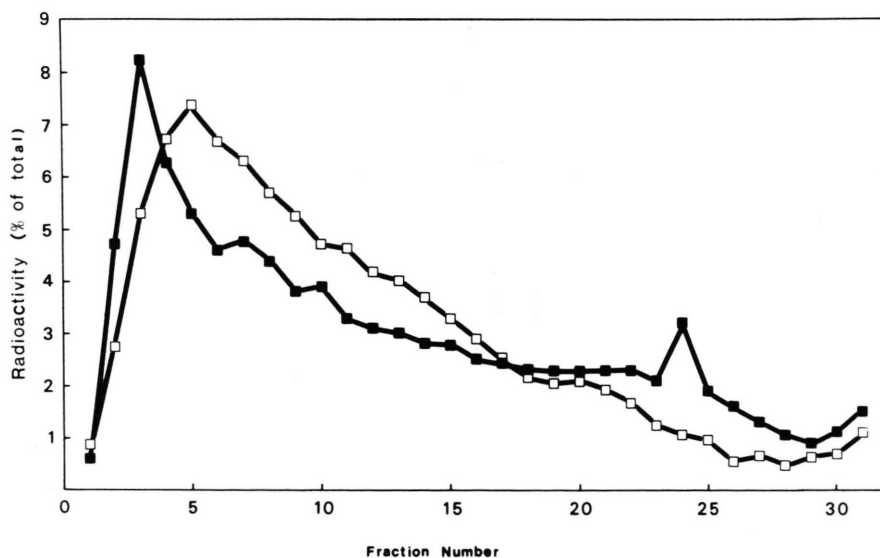


Fig. 5. Alkaline sucrose gradient profiles of DNA from SA 229 (*rec⁺*): ■—■ starved of thymine, □—□ unstarved.

weight DNA than *rec⁺* cells. It is therefore not surprising that the proportion of dead cells found in *rec BC* population is higher than that observed in wild type strains [27]. At present it is difficult to produce a reasonable explanation for the *rec⁺* cells, unstarved for thymine, showing more lower molecular weight DNA than the cells starved for thymine and a search for an explanation is in progress.

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